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Narrative Review

Beyond guidelines: what do I need to know when dealing with fungal diagnostics?

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ABSTRACT

Background: Diagnosing invasive fungal infections (IFIs) is notoriously challenging. Test sensitivity and specificity vary with fungal burden, overlapping microscopic fungal appearances, lack of quantitative culture thresholds and, above all, the patient's immune status, specimen quality and prior antifungal exposure. These variables can mask or mimic disease, leading to delayed or erroneous treatment decisions.

Objectives: This study aims to distil the practical, 'beyond-guideline' insights that clinicians and laboratorians need for reliable IFI diagnosis, and to outline a context-driven approach that complements existing recommendations.

Sources: Narrative synthesis of PubMed-indexed literature on fungal diagnostics published chiefly between 2020 and 2025, augmented by current global guidelines from European Confederation of Medical Mycology (ECMM)/International Society for Human and Animal Mycology (ISHAM)/American Society for Microbiology (ASM) and selected landmark papers outside this window when foundational.

Content: The review explains how (a) host immune state (neutropenia, AIDS, corticosteroid use) skews antigen-vs.-antibody performance; (b) specimen choice (bronchoalveolar lavage, blood, cerebrospinal fluid, tissue) and site of disease dictate test yield; (c) pre-emptive or empiric antifungals suppress culture and antigen signals yet may leave PCR positive; (d) cross-reactivity (e.g. β -D-glucan with bacteraemia, galactomannan with *Fusarium*) and mixed infections cloud interpretation; and (e) colonization must be separated from invasion through combined microbiology, and clinical risk assessment. Traditional microscopy/culture, antigen assays, PCR and emerging next-generation sequencing are compared across major pathogen groups, with tables summarizing sensitivities, specificities and pitfalls.

Implications: Applying an approach that layers multiple modalities according to patient risk and specimen type can shorten time-to-diagnosis, reduce false negatives/positives and enable earlier targeted therapy. Integrating these context-aware steps into routine practice and future guideline updates is likely to improve IFI outcomes and optimize antifungal stewardship. **Cornelia Lass-Flörl, Clin Microbiol Infect 2025;■:1**

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Invasive fungal infections (IFIs) are challenging to diagnose [1]. Recent consensus guidelines emphasize an integrated diagnostic approach, harmonizing traditional techniques (microscopy and culture) with modern diagnostic methodologies (PCR and biomarkers), tailored to individual fungal pathogens [2]. A combination of laboratory methods is often used to improve detection of IFI and medical guidelines provide evidence-based recommendations to healthcare professionals, aiming to standardize clinical practice, improve patient outcomes, ensure quality of care, and guide

clinicians in patient management. Below is an overview of common diagnostic techniques—microscopy, culture, antigen detection, serology, and molecular diagnostics—highlighting their advantages and limitations (e.g. sensitivity, specificity, turnaround time, ease of use), [Tables 1–4](#) [1–9]. Several host and treatment factors as well as specimen-related issues may affect the performance of diagnostic assays ([Table 5](#)). Understanding these factors is crucial for choosing the right tests and interpreting results correctly.

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Table 1
Diagnosis of yeast infections: *Candida* species (data compiled from [1–4])

Clinical specimens
Blood (primary specimen)
Sterile body fluids (peritoneal fluid, CSF)
Diagnostic methods and sensitivity/specificity
Microscopy
Rapid detection, variable sensitivity (~50–60%), high specificity (>90%).
Culture (blood culture)
Sensitivity: ~50–70%, specificity: near 100%.
PCR methods
Multiplex <i>Candida</i> real-time PCR (direct blood testing): sensitivity 84%, specificity 33%
Positive blood culture: panfungal PCR sequencing (from sterile samples): sensitivity ~81%, specificity ~76%
Biomarkers (β-D-glucan): sensitivity ~85%, specificity ~76%

CSF, cerebrospinal fluid.

Table 2
Diagnosis of (mainly) *Aspergillus fumigatus* infections (data compiled from [3–6])

Clinical specimens
Bronchoalveolar lavage (BAL), tissue biopsies, serum
Diagnostic methods and sensitivity/specificity
Microscopy (histopathology)
Sensitivity: ~50–80%, specificity high (>90%)
Culture (BAL specimens)
Sensitivity: ~50%, specificity nearly 100%
PCR methods (BAL and blood)
<i>Aspergillus</i> -specific PCR (ITS/28S rDNA/β-tubulin): sensitivity typically >80%, specificity >90%
Biomarkers
Galactomannan antigen (BAL fluid): sensitivity ~90%, specificity ~95%.
Serum galactomannan: sensitivity ~70–90%, specificity ~85–95%.
Serum β-D-glucan: sensitivity ~75–80%, specificity ~70–80% (not specific for <i>Aspergillus</i> alone)

Table 3
Diagnosis of mucormycosis (Mucorales species) (data compiled from [4,7])

Clinical specimens
Tissue biopsies, BAL fluid, sinus samples
Diagnostic methods and sensitivity/specificity
Microscopy (histopathology):
High specificity (>90%) due to distinctive hyphae, sensitivity moderate (~50–70%).
Culture
Sensitivity: limited (~50%), specificity: nearly 100%.
PCR methods (tissue, BAL):
Mucorales-specific PCR (ITS/28S rDNA/18SrDNA): sensitivity 80–90%, specificity >90%
Biomarkers
No validated biomarker currently available.

BAL, bronchoalveolar lavage.

Here, information is highlighted that can significantly influence diagnostic accuracy but which is not explicitly mentioned in various diagnostic recommendations. This review is confined to invasive fungal diseases—chronic diseases or allergic syndromes are beyond our scope, except to emphasize how their diagnostics differ. In summary, understanding whether a patient is immunocompromised or immunocompetent guides which tests will be most reliable and how results should be interpreted, ensuring laboratory findings are viewed in the proper clinical context.

Patient immune status: immunocompromised vs. immunocompetent hosts

The patient's immune condition heavily influences test accuracy. Immunosuppressed patients (e.g. neutropenic chemotherapy patients, transplant recipients on immunosuppressants, patients

Table 4
Diagnosis of scedosporiosis and lomentosporiosis (*Scedosporium* sp., *Lomentospora prolificans*) (data compiled from [8,9])

Clinical specimens
BAL fluid, tissue biopsies, blood (for disseminated infection)
Diagnostic methods and sensitivity/specificity
Microscopy (histopathology)
Sensitivity variable (moderate), specificity high (>90%)
Culture
Sensitivity: moderate (~50–60%), specificity nearly 100%
PCR methods
Multiplex real-time PCR (ITS-region) in sputum: sensitivity ~62%, specificity 97%
Broad-range PCR (ITS/28S rDNA) on clinical samples: Sensitivity ~68–96%, specificity ~98%
Biomarkers: β-D-glucan (BDG): sensitivity ~81.5%; specificity currently unclear

BAL, bronchoalveolar lavage.

Table 5
Key factors influencing diagnostic accuracy in invasive fungal infections

1. Immune status
 - Affects sensitivity of antigen vs. antibody tests
 - Neutropenic patients → higher GM sensitivity
 - Patients with AIDS → positive antigen, often negative antibody
 - Corticosteroids may lower antigen release
2. Specimen type and site of infection
 - BAL superior to serum for *Aspergillus*
 - CSF essential for cryptococcal meningitis
 - Blood for candidaemia and β-D-glucan (BDG)
 - Tissue biopsy displays reference standard but often not feasible
3. Prior antifungal therapy
 - Decreases culture sensitivity significantly
 - Can lower antigen/PCR yield
 - Serial testing might help to monitor treatment response
 - Must interpret negatives cautiously posttreatment
4. Cross-reactivity and coinfections
 - BDG falsely positive in bacteraemia
 - GM reacts to non-*Aspergillus* fungi
 - Coinfections common in immunosuppressed
 - Serology cross-reacts (e.g. histoplasma/blastomycosis)
5. Colonization vs. invasive disease
 - Candida/Aspergillus* can colonize without causing disease
 - Positive culture is not always indicative for infection
 - Imaging and host factors and multiple tests = diagnosis
 - Clinical context is key

BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; GM, galactomannan.

with late-stage HIV) often have high fungal burdens but a blunted immune response [5]. This paradoxically makes antigen tests more useful (more organism present to release antigen) and antibody tests less reliable [10]. For instance, the *Aspergillus* galactomannan (GM) test actually shows higher sensitivity in patients with neutropenia (who often have extensive angio-invasive growth) than in patients without neutropenia [6]. Fig. 1 [3,7,11] shows how the immune status shapes the course of *Aspergillus* infection (allergic, chronic, airway-invasive and angio-invasive) and how it impacts diagnostic modalities.

In contrast, immunosuppressed patients may fail to produce antibodies—many IFIs in these patients yield negative serology data. A person with AIDS can have disseminated histoplasmosis with a strongly positive urine antigen test but negative antibody titres, whereas an immunocompetent person might show antibodies [12]. In immunocompromised hosts, clinicians rely more on antigen, molecular, microscopy, and culture (and histopathology if feasible), whereas a negative antibody test is unsurprising [3]. On the other hand, immunocompetent patients with chronic or subacute fungal infections often mount detectable antibody responses, making serology useful in those scenarios (e.g. coccidioidal pneumonia in an otherwise healthy person) [3,13]. For instance,

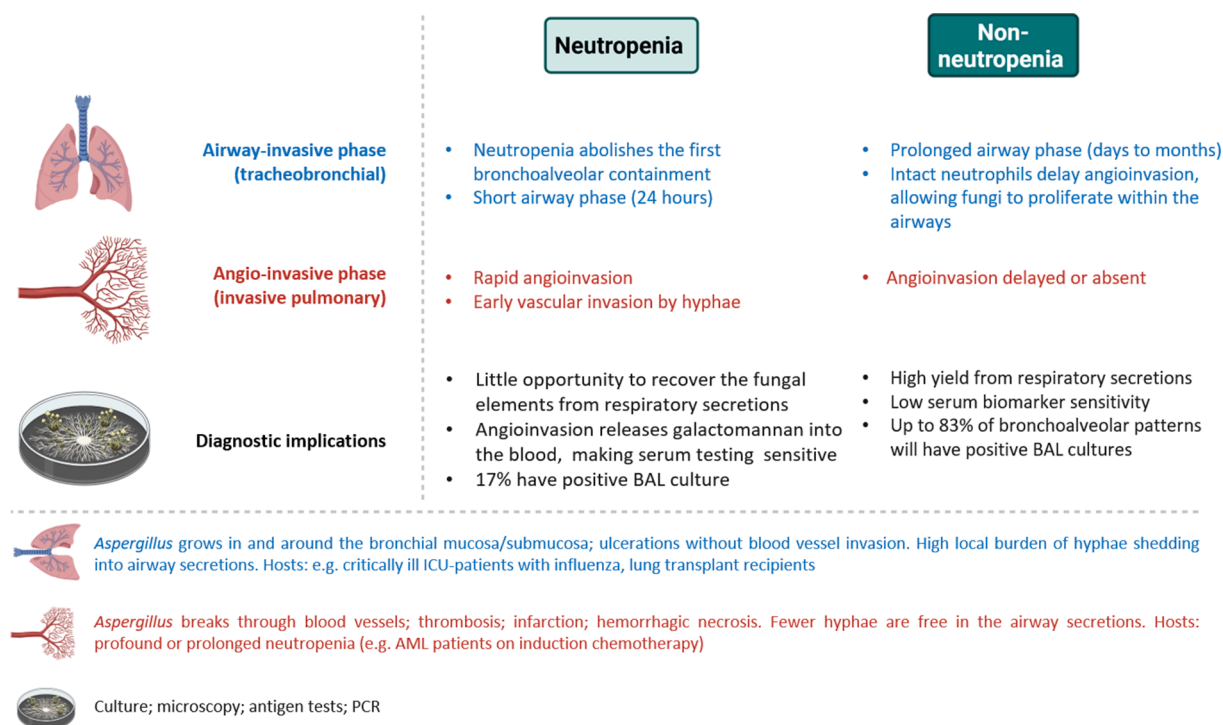


Fig. 1. Evolution of invasive pulmonary aspergillosis based on the immune status and the implication on diagnostic modalities [3,10,11].

IgG antibodies against *Aspergillus* are typically present in chronic pulmonary aspergillosis or subacute invasive aspergillosis, supporting those diagnoses in patients without neutropenia [3,14]. Another example is *Candida* mannan antigen and antimannan antibody assay; these tests target a *Candida*-specific cell wall polysaccharide and the host's immune response to it. Used together, mannan antigen and antimannan IgG/IgM may improve detection of *Candida* infections [3,13]. Neutropenia predisposes to gut translocation of *Candida* into bloodstream. Fungaemia ensues without a prolonged tissue invasive phase, so blood cultures remain the cornerstone, though their sensitivity is only ~50%; additional diagnostic tests become vital [1].

Immune status may also affect the interpretation of positive lab results. An immunocompetent patient who has positive *Aspergillus* culture might be fighting off a colonization more effectively, whereas an immunosuppressed patient with the same results is more likely to have true invasive disease [5]. Steroid use is another factor—patients on corticosteroids may have suppressed inflammation such that they can have significant fungal infection without producing as much antigen in blood (less tissue destruction to release antigen), leading to false-negative blood results [5]. In summary, it is important to know that immunosuppression may skew test performance.

Specimen type and site of infection

The diagnostic yield of each method can vary greatly with the specimen type and the location of infection. Blood is a convenient sample, but not all fungi circulate well in blood [1]. For example, *Aspergillus* seldom produces fungaemia detectable by blood culture, rendering this method largely uninformative except in *Aspergillus terreus* infections where in vivo conidiation may occur [15]. Bronchoalveolar lavage (BAL) or tissue biopsy from the affected site is far more likely to recover the organism or yield a positive antigen/PCR result [8,9,16,17]. On the other hand, blood is

the specimen of choice for beta-D-glucan (BDG) and for diagnosing candidaemia—candidaemia often is detectable by blood culture and blood PCR, whereas noncandidaemic deep-seated candidiasis (e.g. intra-abdominal candidiasis) might require different sampling for best results [18]. Tissue biopsy (with microscopy and culture) is considered the reference standard for many deep-seated infections because it shows invasive disease and allows all methods (microscopy, culture, PCR) to be applied on the same specimen. Obtaining tissue is not always possible due to various host factors such as thrombocytopenia or poor health status. Noninvasive samples like sputum or urine can be useful for certain tests e.g. urine *Histoplasma* antigen is highly sensitive for disseminated histoplasmosis, but a sputum culture growing *Aspergillus* in an immunocompetent patient might just indicate colonization [19]. There are also no quantitative colony count cut-offs in fungal culture; even a single colony from a sterile site is significant, while a high colony count from a nonsterile site does not necessarily prove invasive infection. Cerebrospinal fluid (CSF) is the critical sample for fungal meningitis—fungal culture and cryptococcal antigen in CSF have high yield for cryptococcosis, whereas a blood test alone might miss a Central Nervous System (CNS) infection [19]. The bottom line here is that sending the appropriate specimen for the suspected site of infection increases diagnostic accuracy. BAL for lung infections, blood for disseminated infections, CSF for meningitis, urine for certain endemic fungi, and tissue biopsy when feasible are key considerations [8,20].

Prior antifungal therapy

Starting antifungal treatment before diagnostic sampling can significantly reduce test sensitivity across the board. Culture is most affected—even a single dose of an effective antifungal therapy can render blood or tissue cultures negative by killing or inhibiting the organisms [1,8]. Thus, if possible, it is ideal to obtain

cultures before initiating therapy. If that is not possible, one must recognize a likely decrease in culture yield. Also, antifungal treatment can alter the appearance of fungal hyphae on direct microscopy, complicating the immediate distinction between Mucorales and septate hyaline moulds like *Aspergillus*. Antifungal-exposed hyphae may appear irregular and, if the characteristic branching of septate moulds is poorly visualized, they can be mistaken for Mucorales, leading to potential misidentification [20]. Antigen levels may also drop with therapy [16]. For instance, patients on antifungals have declining GM levels, so a test that might have been positive earlier could become negative after treatment. This is why serial antigen testing is used—a rising or persistently positive antigen can indicate treatment failure, whereas a decline might indicate success [17]. Early therapy can prevent antigen from ever rising high enough to detect. PCR detection might be somewhat more resilient, as it can pick up dead organism DNA, but over time effective therapy reduces fungal burden and thus the DNA target for PCR [7,21]. In summary, pretest antifungal therapy increases the chance of false negatives in culture, antigen, and even molecular tests. When interpreting a negative result, one should ask: Was the patient already on antifungals that could have suppressed the fungus? Conversely, a positive result despite ongoing therapy is very meaningful, and may indicate persistent infection. If a patient has been on prophylactic antifungals (e.g. posaconazole prophylaxis), certain tests like GM might have lower sensitivity [6]. Always inform the lab of any antifungal drugs the patient is receiving, as this context helps judge the reliability of a negative test.

Cross-reactivity and coinfections

The presence of other organisms, fungal or even bacterial, can interfere with fungal diagnostics. It is important to know the test spectra: BDG is a component of most *Candida* and *Aspergillus* cell walls (and also *Pneumocystis* and some others), so a positive value may suggest an infection [3]. BDG is typically negative in mucormycosis and cryptococcosis. Positivity may also occur due to certain bacterial products or contaminants (e.g. *Pseudomonas* bloodstream infections and exposure of blood to glucan-containing gauze can cause false BDG elevations) [13]. GM will be positive in some non-*Aspergillus* fungal infections (e.g. *Histoplasma*, *Fusarium*, *Penicillium*, *Saprochaete*, *Paecilomyces*, and *Trichoderma* species). Clinicians should be aware of such results to avoid misdiagnosis—a positive GM in an immunosuppressed patient with skin lesions might indicate *Fusarium* as well as *Aspergillus*. Historical reports of GM reacting with an *E. coli* polysaccharide are another example for cross-reactivity [3].

If a patient has multiple fungal infections, which is less common but possible in highly immunosuppressed patients, one antigen test might respond to both. Cross-reactivity can also occur in serology; e.g. *Blastomyces* and *Histoplasma* antibody tests may cross-react due to shared antigens, so travel history and other clues must be used to discern the likely agent [21]. It is also important to consider that in certain clinical scenarios, two invasive fungal processes could coexist. For example, a patient with AIDS could have *Cryptococcus* and *Histoplasma* simultaneously, or an Intensive Care Unit (ICU) patient could have candidaemia and aspergillosis and/or *Pneumocystis* infection together [22]. In such cases, one test does not rule out the other infection; multiple tests might be positive. Additionally, nonfungal infections can cause misleading results. Clinicians must be aware of these possibilities to avoid misdiagnosis. Contaminating flora in samples can also complicate culture/PCR—e.g. a colonized airway specimen will grow various moulds on culture that might not be the true pathogen [19]. Proper sampling, e.g. a sterile tissue biopsy vs. a swab of a superficially colonized area

mitigates this. In summary, cross-reactivity can reduce specificity, and coinfections can confuse the clinical picture and fungal test results. Understanding test characteristics, such as specificity, sensitivity, and cross-reactivity, is key. When an unexpected positive result arises, consider whether it could be a cross-reaction or if it points to an alternate infection.

Colonization vs. infection

Many fungi like *Candida* and *Aspergillus* species can colonize humans without causing invasive disease, especially in the respiratory and gastrointestinal tract [5]. Diagnostic tests might pick up colonizing organisms [3]. A prime example is a positive culture of *Candida* from BALs that reflect colonization and not true infection in most of the cases. For *Aspergillus*, as noted, isolating the fungus from sputum in an immunocompetent patient often indicates colonization, whereas in a patient with neutropenia it likely indicates invasive aspergillosis. Distinguishing colonization from infection often requires multiple lines of evidence, e.g. clinical signs, underlying disease, radiology, and multiple positive tests. Laboratories often flag potential contaminants—e.g. *Penicillium* grown from a single sputum sample may be reported as a possible contaminant and should be interpreted in the clinical context [19]. Similarly, routine practice advises confirming a mould isolate by obtaining the same species from a second sputum culture to exclude contamination. These examples remind us that diagnostic specificity in practical terms depends on context; a test may be technically 'positive' for fungus, but that fungus might not be the culprit of disease. Combining results, such as antigen plus culture plus imaging, and assessing host risk factors improves diagnostic accuracy for true invasive disease. It is important to stress that this stepwise diagnostic approach—integrating multiple methodologies and accounting for host factors—is precisely what current guidelines strongly endorse.

Key diagnostic modalities at a glance: current and emerging

Traditional diagnostics remain the reference standard for a definitive diagnosis of invasive fungal disease, but they suffer from low sensitivity and slow turnaround [1,3,13]. Direct microscopy and histopathology offer rapid, presumptive identification of fungal elements (e.g. ribbon-like aseptate hyphae in mucormycosis) but cannot speciate or assess susceptibility [20]. Any growth from a normally sterile site is diagnostic, yields the organism for species identification and resistance testing. Serological assays function as adjunctive tests, may detect infections earlier than culture but have cross-reactivities and thus require careful clinical interpretation. In recent years, molecular methods have rapidly advanced, improving early detection from blood and tissue samples (Tables 1–4) [1–9]. PCR assays allow direct detection of fungal DNA/RNA with high analytical sensitivity. A key advantage is that PCR does not require viable organisms—even DNA from dead fungi can be amplified—so prior antifungal therapy has less immediate impact on PCR than on cultures [4]. Despite these advances, molecular diagnostics face challenges. Fungal cells have tough cell walls (chitin, glucans) that make nucleic acid extraction difficult, and clinical specimens often contain low fungal DNA burden amid abundant host DNA [4]. This can lead to false negatives if sample processing is not optimized. On the flip side, PCR is extremely sensitive and can pick up DNA from contaminants or colonizing fungi, so false positives are a concern, especially in nonsterile samples [4]. Beyond PCR, next-generation sequencing (NGS) approaches are starting to influence fungal diagnostics. Metagenomic NGS can detect DNA of virtually any pathogen in a clinical sample without prior suspicion [4]. This high-throughput technology has successfully identified unexpected fungi in cases where routine tests were negative. At present, NGS serves

as a valuable adjunct for difficult cases (e.g. culture-negative infections or polymicrobial scenarios) and as a research tool, rather than a first-line diagnostic. The main drawbacks include high costs, infrastructure, slow turnaround (24–72 hours), overwhelming host–DNA background, contamination risks, inconsistent bioinformatics and database gaps, limited data on viability or resistance [4]. As sequencing becomes faster and cheaper, we anticipate broader clinical adoption.

In conclusion, diagnosing IFIs requires a balanced use of methods. Microscopy and culture offer specificity and the possibility of definitive diagnosis, hence display the reference standard, but suffer from low sensitivity and slow time to result [20]. Antigen and molecular tests improve early detection and sensitivity, at the cost of some specificity and availability issues [16]. Serology is invaluable for certain infections and patient groups but useless in others. No single test is sufficient in many cases; thus, clinicians often employ multiple tests in parallel (e.g. culture plus antigen plus PCR) to increase diagnostic yield. Although *A. fumigatus* is the most common aetiological agent of invasive aspergillosis, other species like *A. flavus*, *A. terreus*, and *A. niger* are also clinically significant [17]. Diagnostic strategies for nonfumigatus *Aspergillus* species share the GM cell wall antigen, so the GM test will typically be positive in invasive infections. Likewise, BDG will be elevated in invasive infections by any septate mould including *Aspergillus*, because BDG is a common cell wall polysaccharide. Culture and sequencing remain important for nonfumigatus *Aspergillus* because identifying the species can influence therapy.

The choice of test is guided by the clinical context and covers the patient's immune status, suspected fungus, site of infection, and timing. Understanding each method's pros and cons as well as the factors affecting performance helps optimize the diagnostic approach. Early and accurate diagnosis is critical, as timely targeted antifungal therapy can be life-saving in invasive mycoses. The ongoing development of improved diagnostics (like rapid PCR assays, NGS, and better antigen/antibody tests) holds promise to further enhance our ability to detect these challenging infections.

CRedit authorship contribution statement

Cornelia Lass-Flörl: Writing- Original draft preparation, Proofreading.

Transparency declaration

Potential conflict of interest

The author declares that they have no conflicts of interest.

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During the preparation of this work, the author used GTP-4 (OpenAI©) to improve readability, and in addition to prepare

Tables 1–4. After using this tool/service, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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